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AN EXPERIMENTAL STUDY OF BONE GROWTH AND THE SPINAL BONE TRANSPLANT *

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NEW YORK

The special object of the animal experimentation reported herein was to afford means for studying both macroscopically and microscopically a bone-graft when planted in a dog by precisely the same technic as that which I employ in the human subject for the treatment of Pott's disease of the spine.[1]

I wish to express my thanks to the following for valuable aid rendered in this work:

Dr. Ferguson for his interest in superintending the preparation of most of the microscopic sections, for his examination of these sections and for many valuable suggestions.

Dr. Beebe for aid, suggestions and laboratory privileges.

Drs. Soule and Keller for indispensable aid in the operating-room.

REPORT OF EXPERIMENTS

Experiment 1.—Dec. 10, 1911: Dog, mongrel, male, approximate weight 30 pounds. Ether anesthesia. Anesthetist, Mr. Cassellius. Fields of operation: dorsal region of back and left fore leg, prepared by shaving, and scrubbing with tincture of green soap and water, followed by corrosive sublimate 1:1,000, and 70 per cent. alcohol. The spinous processes of three of the mid-dorsal vertebrae were approached by an incision through the skin and areolar tissue directly over their tips. The supraspinous and interspinous ligaments were split with a scalpel to a depth of two-thirds of an inch between the spinous processes without disturbing the attachments of the

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* From the Loomis Laboratory, Cornell University Medical College.

[1] Albee, Fred. H.: Transplantation of a Portion of the Tibia into the Spine for Pott's Disease, The Journal A. M. A., Sept. 9, 1911, p. 885; Bone transplantation as a Treatment for Pott's Disease, Club Feet and Ununited Fractures, Post-Graduate, November, 1912, p. 999; New York Med. Jour., March 9, 1912. Other studies in bone growth, periosteal transplantation, etc., have been carried on, coincidently, as will appear.

ligaments to the spinous processes. Each of the three spinous processes was split longitudinally with a chisel and mallet into halves for a depth of about two-thirds of an inch, care being taken that the right halves of the spinous processes were not broken. A separation of the tips of the halves of these spinous processes produced a wedge-shaped cavity, into which the ulnar transplant was later inserted. A hot saline compress was placed in the wound until the bone insert was obtained. This was for the purpose of securing as perfect hemostasis as possible for a bed for the bone-graft. Half the diameter of the shaft of the dog's right ulna was then removed with chisel and the bone-forceps. The graft consisted of periosteum, compact bone, endosteum and marrow substance. It was inserted between the halves of the interspinous ligaments

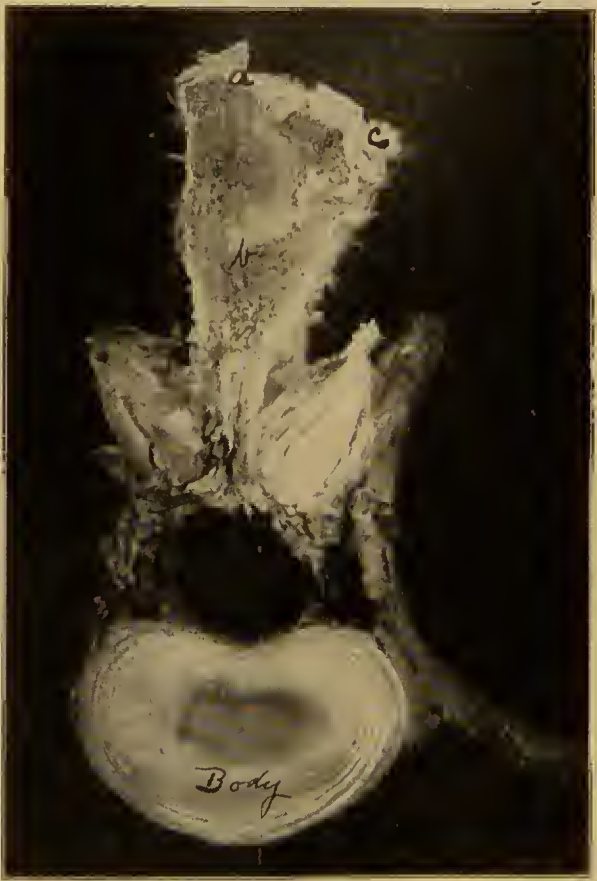

Fig. 1.—View of a dog's vertebra (Experiment 1), into the spinous process of which a portion of his ulna had been ingrafted six months before; *a*, *b* and *c* indicate the outlines of the graft, which has become firmly grown into the split spinous process. Figure 2 is from a photomicrograph of a section of graft grown into the spinous process at this point.

and spinous processes, and held in place with interrupted sutures of linen, which were passed through the supraspinous ligament and the posterior edge of the halves of the interspinous ligaments near the tip of each spinous process. These ligaments were thus drawn over the graft posteriorly. The procedure was precisely that which I have applied to human subjects and of which I have published reports.[1]

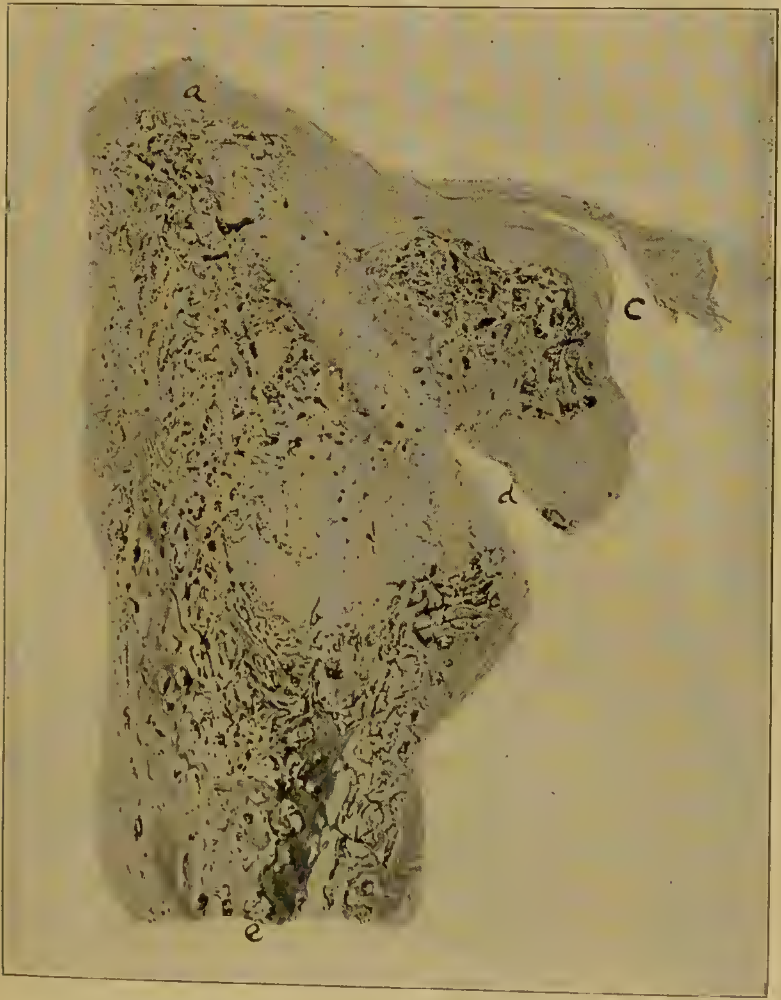

Fig. 2.—Decalcified section through long axis of spinous process with cross-section of the grown-in graft. Six months after a portion of same animal's ulna had been grafted into spinous processes. A careful microscopic study of these sections and all others have failed to disclose dead bone-cells. The corners of the graft are indicated by *a*, *b* and *c* (*d* is a microtome artefact); *e* is base of spinous process.

December 13, the wound was septic. December 20, much pus was discharging from the wound. Sinus slowly decreased in size in the next few weeks.

Necropsy.—May 28, 1912: The wound had healed with the exception of a small sinus; a sliver of nearly one-third the diameter of the graft anteriorly and tapering to a pointed end posteriorly had sequestrated from the rest of the graft, which had become firmly united to the spinous processes. The whole posterior diameter had lived and become firmly grown into the spinous processes. The result was a bridge of bone uniting three vertebrae. Although sepsis had occurred and no attempt had been made to immobilize the dog's spine, a gauge dressing was applied at time of operation and the dog was placed in a cage. X-ray examinations of the gross specimen and a microscopic study of decalcified and non-decalcified sections failed to show degeneration of that part of the graft which had become united. No cartilage cells could be found; the union of graft to spinous process was by new bone formation.

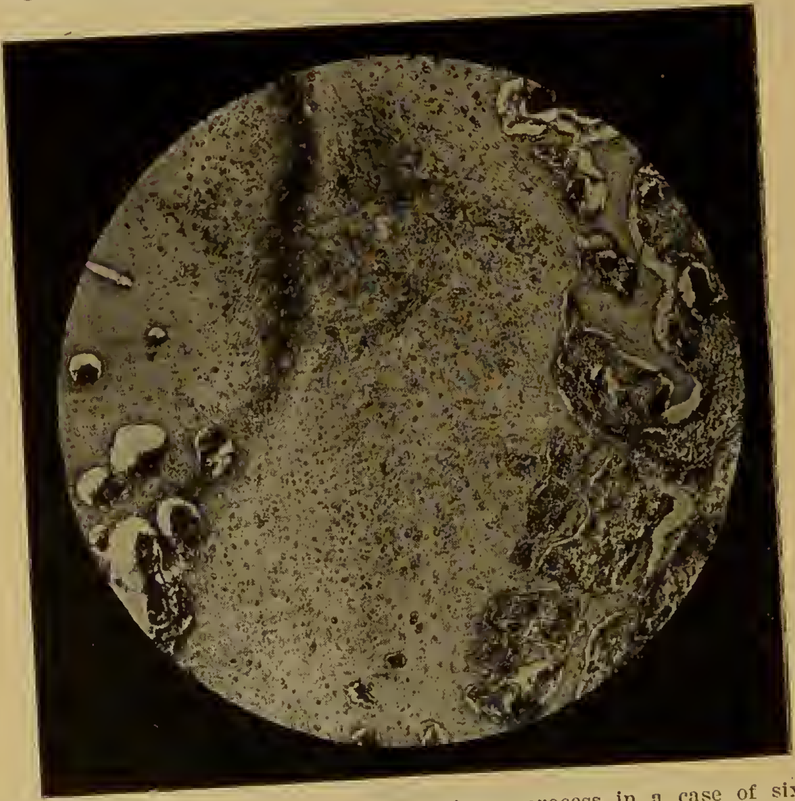

Fig. 3.—Junction of graft and spinous process in a case of six months (Experiment 1). No dead bone-cells could be found in these specimens. From a high-power photomicrograph.

EXPERIMENT 2.—Jan. 18, 1912: Dog, male, mongrel, weight 20½ pounds. Ether anesthesia. Assistant, Dr. Keller. Precisely the same technic was carried out in this case as in Experiment 1, except that the whole diameter of the shaft of the ulna was removed subperiosteally. A sharp periosteum elevator was used and the periosteum was removed from the bone with force for the purpose of being certain of separating the deep osteogenetic layers from the bone cortex and obtaining it as a part of the periosteum. The periosteum was left *in situ* in the leg. The transplant was inserted by the same technic as in Experiment 1, except that the periostum had been removed as previously described. The leg and back wounds were closed by a continuous suture of linen. The periosteal tube was allowed to collapse.

Jan. 22, 1912: Back wound septic.

Jan. 28, 1912: Leg wound healed by primary union.

Necropsy.—Feb. 29, 1912: Back wound was septic; graft had sequestrated. It is believed that the difficulty in preparing the dog's skin for operation, in addition to large hemotomata which invariably collect into loose areolar tissue of the dog's back in spite of all precautions, was largely responsible for the sepsis in the back wound, at the same time the leg wounds were clean.

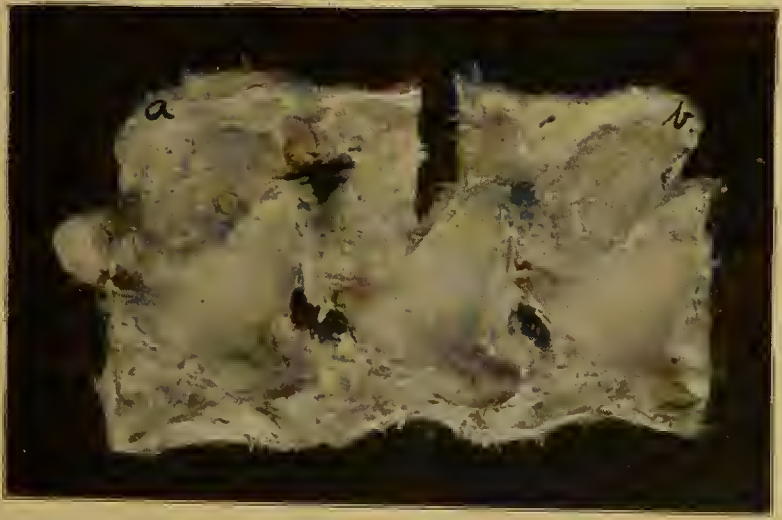

Fig. 4.—Specimen from Experiment 1 showing bridge of bone between three vertebrae, the result of a bone-graft inserted six months before.

The conditions about back wound were precisely the same as those in Experiment 1, namely, a deposit of osteoid tissue in wall of sequestrum pocket. The graft, however, had sequestrated.

In the leg a bridge of bone had appeared where the shaft of the ulna had been removed. In the center it tapered to a diameter about one-half that of the original bone. This bone growth had been very rapid and connected the ends of the ulna.

Experiment 3.—Jan. 23, 1912: Dog, terrier, female, weight 22 pounds. Ether anesthesia. Anesthetist, Mr. Cassellius.

Assistant, Dr. Keller. The spinous processes of last two dorsal and first, second and third lumbar vertebrae were prepared as usual for the graft. With chisel and mallet and saw about 1¾ inches of the left ulna were removed leaving the periosteum, from one-half its diameter, *in situ* in the leg. The periosteum was removed in this instance with the blunt end of a pair of scissors and no force was used in scraping the periosteum from the bone. The periosteum peeled off easily. The portion of the ulna which had been removed was then split longitudinally with a chisel into equal parts, on one of which was the periosteum. This latter fragment was inserted into the last two dorsal vertebrae. The portion from which the periosteum had been removed was inserted into the second and third lumbar vertebrae.

Necropsy.—Feb. 29, 1912: Posterior end of wound was filled with pus. Anterior end of wound had healed by granulation. The graft from which the periosteum had been removed and which had been placed in the lumbar vertebrae had sequestrated and was surrounded with pus. The graft insert which was placed in the dorsal vertebrae was firmly united into those vertebrae. A microscopic examination showed the union to be bony. No evidence of degeneration or cellular death could be found. No bone or evidence of osteogenesis appeared between the ends of the ulna where the grafts had been obtained, except about the bone ends. The periosteum in this case, as stated, was removed from the bone by means of a blunt instrument and with no effort to get into a deep cleavage.

EXPERIMENT 4.—Jan. 25, 1912: Dog, mongrel, female. Ether anesthesia. Anesthetist, Mr. Cassellius. Assistant, Dr. Soule. This dog on the evening before the operation received a bath in 2 per cent. aqueous dilution of liquor cresolis compositum. In all following experiments this bath was given. Two inches of the shaft of left ulna were removed subperiosteally. A sharp periosteal elevator was used for the purpose of getting into deep cleavage. The portion of the ulna shaft removed was then split longitudinally with a chisel and mallet into halves. One of the bone fragments was inserted by the usual technic into the last dorsal and first and second lumbar vertebrae. The remaining bone fragment was placed in sterile normal salt solution in an ordinary ice-box for the purpose of transplanting it into the next dog operated on. Wounds healed by primary union.

Necropsy.—May 9, 1912: Transplant was found united firmly with the spinous processes. The graft had lost its identity. From the appearance of the bridge of bone between the tips of the three spinous processes one could not say from what it had originated. Microscopic examination showed bony union of graft to spinous process and no evidence of cellular degeneration. A strong bridge of bone was present between ulna ends filling in the space where the transplant had been removed. This bridge of bone was not quite so large in diameter as the normal ulna shaft. It was, however, sufficiently strong for functional purpose. It is believed that the satisfactory osteogenesis obtained in this case as, for instance, compared with practically no bone growth in Experiment 3, was due to the method of removing the periosteum by a sharp instrument for the purpose of getting into deep cleavage. In this way the chances are large that the very active osteogenetic layer of cells which is situated on the surface of and considerably adherent to the compact bone, will be loosened either entirely or partly, and separate off as a part of the periosteum.

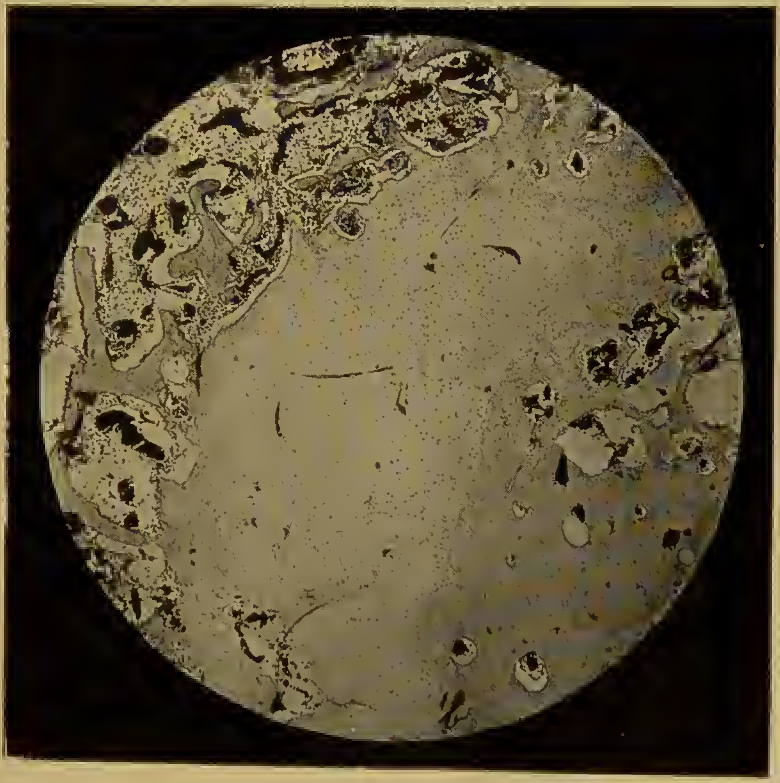

Fig. 5.—Junction of graft and spinous process (*a*, *b*). The transplant had been inserted four months (Experiment 5). From a high-power photomicrograph.

Experiment 5.—Jan. 30, 1912: Dog, black hound, male. Ether anesthesia. Anesthetist, Mr. Cassellius. Assistant, Dr. Soule. Incision was made over last three dorsal and first three lumbar vertebrae. Spinous processes of the three lumbar vertebrae were split into right and left halves with a chisel and the bone fragment 2 inches long which had been removed from the dog's ulna in Experiment 4, Jan. 25, 1912, and kept in normal salt solution in an ice-box, was inserted into the prepared lumbar vertebrae. The spinous processes of the tenth and eleventh dorsal vertebrae were denuded with a blunt instrument of their periosteum from the neural arches posteriorly to the tips of the spinous processes where they were left attached to each. The separated ends were then held in approximation by linen sutures. The skin was sutured by the subcutaneous method. In all previous cases the skin was closed with the ordinary through-and-through stitch; all succeeding wounds were closed with the subcutaneous suture, resulting in much less skin infection.

Necropsy.—May 8, 1912: The transplant was amalgamated into the spinous processes and had nearly lost its identity. It showed the contour of the graft on one end. From the appearance of the bridge of bone, one would not have thought that it was due to a bone transplant. There were only a few small disconnected plaques of proliferated bone from the periosteum which had been stretched across between the dorsal spinous processes.

It is thought that the fact that there was not a satisfactory bone growth from the periosteum may be accounted for in two ways: first, the periosteum on the spinous processes is meager and unsatisfactory on account of so many muscular and ligamentous attachments; secondly, the periosteum was removed with a blunt instrument without a special effort being made to get into deep cleavage.

Experiment 6.—Jan. 30, 1912: Dog, bull, male, weight 28 pounds. Ether anesthesia. Anesthetist, Mr. Cassellius. Assistant, Dr. Soule. Incision was made over and down to spinous processes of last two dorsal and first three lumbar vertebrae. Two and one-fourth inches of left ulna with its periosteum were removed with saw and split with chisel longitudinally into halves. These fragments of bone were then inserted into the spinous processes by the usual technic, one into the lumbar vertebrae, the other into the lower dorsal. The wounds healed by primary union.

The dog was found dead May 6, 1912. In my absence Mr. Cassellius removed the operated portion of the spine and placed it in fixing solution for future examination. These transplants, like all the others in the presence of asepsis, had become firmly united to the spinous processes into which they had been inserted.

Experiment 7.—Feb. 1, 1912: Young dog, mongrel terrier, weight 18 pounds. Ether anesthesia. Anesthetist, Mr. Cassellius. Fields of operation prepared by iodin method. An incision over last three dorsal and first three lumbar vertebrae was made. The lumbar spinous processes were split longitudinally on the left side of their center down to the neural arches. Wound as usual was packed with hot saline compress. One and three-fourths inches of diaphysis of left ulna were removed subperiosteally. A blunt instrument was used to separate the periosteum which was left *in situ* in leg. One-half of the ulna shaft was placed by usual technic into the posterior lumbar vertebra. Pieces of periosteum stripped, without aid of sharp instrument, from ulna (varying in size from one-fourth by one-fourth of an inch to small bits) were sutured into the belly of the muscle, which had been turned aside, when the ulna shaft was removed. The muscle was then sutured into its normal position. The periosteum, on the left sides of the two anterior spinous processes was stripped and retained in two pieces with some difficulty. These periosteal flaps were then drawn together and sutured, thus producing a periosteal bridge between these processes. The remaining half of the ulna was placed in salt solution for the purpose of transplanting it into the next dog operated on.

Necropsy.—At necropsy three months later the transplant was firmly united into the spinous processes. No osseous tissue could be found as a development from the periosteal bridge between the spinous processes. Only a small plaque of new bone could be found where the ulna had been resected. There was new growth of bone about cut ends of ulna.

Experiment 8.—Dog. Ether anesthesia. Anesthetist, Mr. Cassellius. Assistant, Dr. Soule. The last three dorsal and first three lumbar spinous processes were split as usual. Numerous slivers of the previous dog's ulna devoid of periosteum were placed between the split portions of the two upper dorsal vertebrae. The superspinous and interspinous ligaments were drawn over in the same way as when a large graft was used. All the ligaments and muscles were separated from the second and third lumbar spinous processes for about two-thirds of an inch from their tips. These two spinous processes were then split longitudinally into equal anterior and posterior portions. Green-stick fractures were produced in the anterior half of the posterior process and the posterior half of the anterior process. The tips of these fragments, well denuded of periosteum and soft tissues, were then contacted and held with a linen ligature. The ligaments and fascia were drawn over all with a continuous linen suture. Skin was closed as usual with subcutaneous continuous suture.

Necropsy.—At necropsy two and one-half months later there was evidence of some skin infection which had nearly subsided. The slivers of bone had united and produced a bridge of bone between the spinous processes but not so firm as one resulting from one large graft. There was no bony union between the approximated fragments of the second and third lumbar spinous processes.

Experiment 9.—Dog, terrier, male, weight 18¼ pounds. Preparation: bath in compound solution of cresol and shaving the night before operation. Field of operation allowed to dry over night and treated with American tincture of iodin just before operation. This preparation was used in all of the succeeding cases and was found very satisfactory. Ether anesthesia. Assistant, Dr. R. E. Soule. Site in back for graft was prepared as in previous cases and packed with saline compress. Two inches of middle of left ulna were removed subperiosteally with blunt dissector. This portion of the ulna was then split longitudinally into quarters, two of which were placed in the tips of three lumbar vertebrae. A strip of periosteum one and one-fourth inches by one-third inch taken from the ulna by a blunt dissector was placed into the tips of two split spinous processes anterior to those that contained the graft previously inserted. The periosteum was placed in a similar manner to that of the bone graft. The back wound was closed. Two pieces of bone one-fourth inch by one-fourth by one-eighth inch in diameter, which had been taken from the tip of a spinous process of the same dog, were placed in the belly of a muscle in the left fore leg. Two pieces of bone of the same size were taken from ulna and placed in belly of another muscle in left fore leg, the object being to determine which was the more osteogenetic.

The dog was found dead Feb. 27, 1912.

Necropsy.—Feb. 28, 1912. Assistant, Dr. Soule. Bone transplant was found firmly united by callus to those spinous processes into which they had been inserted. The graft presented every appearance of being live bone. The small piece of bone from the tip of a spinous process, which had been implanted into a muscle belly at operation, had changed very little; there was a very small proliferation on the side of the periosteum. The piece of bone taken from the ulna and inserted into a muscle belly had changed also very little. There was, however, considerable proliferation on the side of the periosteum. The piece of periosteum (1¼ inches by one-third inch), which had been placed between the tips of two split spinous processes, presented no evidence of proliferation and was hard to find.

EXPERIMENT 10.—Feb. 13, 1912: Puppy, terrier. Ether anesthesia. Assistant, Dr. Soule. Incision 5 inches long was made over and down to tips of last dorsal and first three lumbar vertebrae. Interspinous ligaments were split with scalpel, spinous process with chisel. A piece of the ulna bone of the dog previously operated on (1¾ inches long and one-half the diameter of the ulna) was inserted by technic above described into the spinous processes of the second and third lumbar vertebrae. A piece of the same dog's left ulna of about the same size was then removed subperiosteally with chisel and saw and inserted into the last dorsal and first lumbar vertebrae. A piece of periosteum (1¼ inches by one-third inch) removed by blunt dissection from the ulna was placed into the fascia one and one-half inches to left of the first lumbar vertebra and fixed with linen sutures. On the opposite side (right) and in the same relation to the first lumbar vertebra a piece of the left ulna (one-half by one-eighth of an inch) was placed in the same manner as the periosteum. The right fore leg was broken over the side of a table and a splint was applied, in order to obtain thickened proliferating periosteum for grafting purposes in ten days' time.

Necropsy.—Feb. 27, 1912: Both pieces of bone, the one from another dog and the one from the same dog, were equally firmly united into the spinous processes. A careful microscopic examination of sections made through graft at various places and through junction of union of graft to spinous process failed to show any evidence of degenerated bone-cells. The periosteum inserted into the fascia to the left of the spine, presented no evidence of proliferation, either periosteal or bone. The bone placed under similar condition to the right of the spine showed considerable proliferation, especially on the periosteal side.

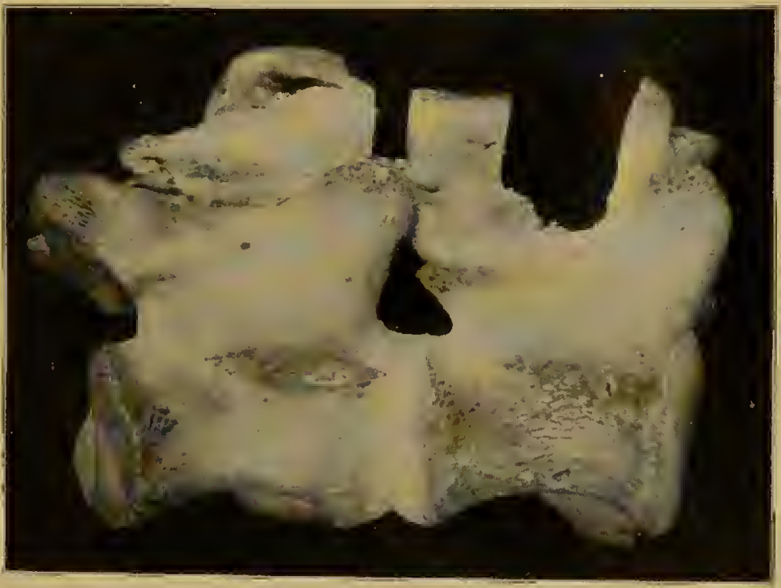

Fig. 6.—Two vertebrae of a dog into which the bone transplant (A, B) had been inserted six weeks before. (Experiment 11); from a photograph. Sections have been taken for microscopic examination.

Experiment 11.—Feb. 15, 1912: Puppy, male mongrel, weight 17¾ pounds. Ether anesthesia. Assistant, Dr. Soule. Shaft of left ulna was removed, with its periosteum intact, and placed in normal salt solution, to be kept in ice-box for a following case. The spinous processes, last dorsal and first lumbar vertebrae were prepared as usual and a piece of a puppy's ulna (one-half its diameter and 1¾ inches long), which had been removed two days previously and kept in

Ringer's solution in an ice-box, was inserted by usual technic into the second and third lumbar vertebrae. A fragment of ulna, with periosteum of approximately the same size, which had been removed from an old dog two days previously and kept in salt solution in an ice-box, was inserted into the last two dorsal vertebrae.

Necropsy.—Six weeks from time of operation: Both bone-grafts were found firmly united into the spinous processes. *X*-rays taken in different planes of specimens (see Fig. 6) failed to show any areas of degeneration in the graft. Both decalcified and non-decalcified (ground) microscopic specimen (see Fig. 7) were prepared and examined carefully. No dead bone-cells could be found. The graft, although only six weeks after insertion, was very completely united into the spinous processes by new-formed bone. No cartilage cells could be found.

Experiment 12.—Feb. 20, 1912: Dog. Ether anesthesia. Anesthetist, Mr. Cassellius. An incision 3 inches long was made over three lower dorsal spinous processes. The muscles and ligaments with periosteum were separated from them down to the neural arches and retracted. The processes were divided at their base with bone forceps and chisel close to the neural arches. The processes were then placed longitudinally so that the tip of one process came into approximation with the severed base of the next superior process. The soft tissues with periosteum were brought back with interrupted sutures of linen. Skin was closed with subcutaneous linen suture. The second and third lumbar processes were then exposed. Their tips were split for two-thirds of an inch *in situ* with supraspinous and interspinous ligaments undetached in the usual manner. A fragment of an ulna removed from a dog one week previously and kept meantime in normal salt solution in an ice-box was inserted as a graft by the usual technic. This transplant was 1¾ inches long and one-half the diameter of the ulna shaft. The skin was closed by subcutaneous suture of linen.

Necropsy.—Two and one-half months after operation: Bony union had not occurred between the dorsal vertebrae whose spinous processes had been broken down. It should be stated, however, that, as in all other cases, no attempt to fix or immobilize the dog's spine was made. The graft which had been inserted into the second and third lumbar processes was well united.

Experiment 13.—April 24, 1912: Dog, mongrel, male, weight 21½ pounds. This experiment was possible only through the kindness of Drs. Beebe and Berkeley, who allowed me to remove a part of a sheep's ulna while Dr. Berkeley was operating on it. A fragment of ulna 3½ inches by one-third inch, including periosteum as well as marrow substance, was removed from a large sheep and placed in normal salt solution until the dog's spinous processes, three in number (first dorsal and first and second lumbar), could be split and prepared in the usual way. The transplant was then inserted and covered with the ligaments as above described.

Necropsy.—Five weeks after operation: Although the wound had healed kindly by primary union, the transplant was found in a pocket filled with serum. No evidence of osteogenesis on the part of the graft or union to spinous processes was present.

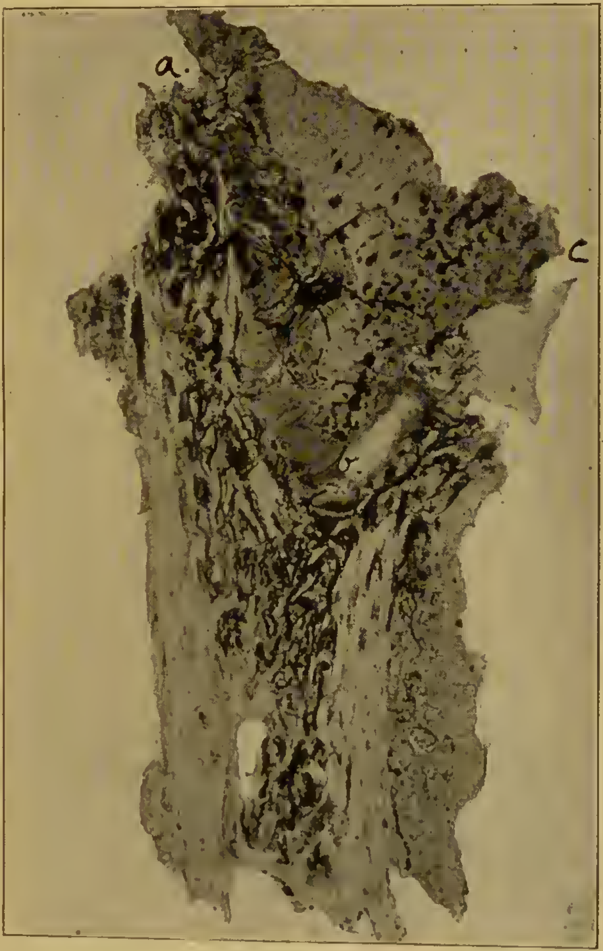

Fig. 7.—Longitudinal section through spinous process with cross-section of graft (*a*, *b*, *c*) which had been inserted six months; from a low-power photomicrograph of a non-decalcified ground specimen. Numerous blood-vessels can be seen, under high magnification, extending from spinous process into graft.

The following deductions and conclusions are based on the experimental work reported herein, in conjunction with a clinical experience gained from 130 bone-grafting operations on the human subject, ninety-five of which have been for Pott's disease, ten for clubfoot, eleven for paralytic deformities, eight for ununited fractures, one for osteomyelitis cavity, one fixation of tarsus for tuberculosis, one for deformity of tibia, one for deformity of the jaw, one for paralytic drop-wrist, and one for absent head and neck of femur and ununited fracture of neck of femur. In three of the cases of Pott's disease, the bone transplant has been cut down on for minor reasons, inspected and parts removed for examination. The time after operation has been three, five and seven weeks. In all of these cases the grafts have been well united into the spinous processes wherever contacted. In two cases the end of the graft had sprung posteriorly into the soft tissues. In every instance the portion of the graft, which was contacted into the spinous processes bled wherever cut with bone forceps; its periosteum was proliferated and the transplanted portion in every way presented the appearance of viability. On the other hand, in both cases, the portion of the graft projecting into the soft tissues was pale, the periosteum had not proliferated, the bone did not bleed in its central portion when cut, and was thought to be acting as an "osteoconductive scaffold," whereas there seemed every reason to believe, especially in the light of our microscopic results, that the portion in and between the spinous processes was viable, on account of the contacted points of haversian blood-supply being so numerous and near together.

SUMMARY

1. Many liberties can be taken with the bone-graft without interfering with its success. It has certain bacteria-resisting properties. In one case the transplant was kept in normal salt solution in an ice-box for one week and in others for shorter periods and successful results followed. In two cases sepsis occurred in the same wound; nevertheless, parts of the graft in each case became united to the recipient bone and remained, while the rest of the transplant sequestrated. From these experiences and the fact that in my series of 130 bone-grafting procedures on human subjects only parts of four grafts sequestrated, it is believed that one is safe in deducting that the bone-graft has considerable germ-resisting ability.

2. It seems very probable that the amount of haversian blood-supply is in a very large degree, if not wholly, responsible in determining whether the bone-graft lives

as such or acts as an osteoconductive scaffold. This was especially emphasized in the three human cases cut down on. If the graft is to live as such the blood-supply contacts must be of favorable character and numerously distributed at short intervals along its whole extent. Such as is the case with the spinal graft or the transplant used for ununited fractures described elsewhere.

3. Ollier in 1858 described his technic for subperiosteal resection, but as far as I am aware, did not emphasize the importance of the employment of a sharp instrument with force in order to separate with the periosteum the embryonic layer of active osteogenetic cells, which is situated on the periphery of the compact bone, although it is evident from the description of his work that he frequently practiced this technic. It seems certain that osteogenesis on the part of the healthy periosteum removed from a healthy bone, is largely dependent on the presence of these active embryonic bone cells from the outer surface of the cortical bone. Therefore the wisdom of the use of the sharp periosteum elevator in bone resection is apparent.

4. The bone transplant apparently acts always as a stimulant to osteogenesis on the part of the bone into which it has been implanted.

5. The spinal graft in the dog loses its identity at about the fourth month. After that time one would not know from its appearance that the bone bridge had originated in this way.

6. Bone taken from another species, such as the sheep, did not unite to the recipient bone of the dog, although in the presence of asepsis. This one experiment does not prove that sheep's bone will not unite to dog's bone as a graft, but it does prove the unreliability of the procedure.

7. A bone bridge between different vertebrae was accomplished in this small series of experiments only by the bone-graft. Breaking down the spinous processes, splitting the spinous processes with approximation to the contiguous halves, and the insertion of periosteal bridges all failed to produce the desired continuous bone bridge.

8. Bone transplants taken from a long bone such as the ulna showed evidence of greater osteogenesis than when taken from the spinous processes.

9. Bone from which the periosteum had been removed proved equally satisfactory to bone-grafts on which the periosteum had been retained, but their persistency was not tested because the animals were not allowed to live long enough. It seems certain that the fate of a bone-graft depends largely on its exact environment especially as to the numerous bone contacts closely situated.

10. The above-mentioned germ-resisting property of the bone-graft, in addition to its early adhesion, by bone growth, to the bones, with which it is contracted, favors in my opinion its substitution when feasible in place of all metal internal splints, especially when it is considered that the metal splint has absolutely the opposite influence, namely, the production of bone absorption, and favors infection.

11. It also seems that it is largely a question of definition of what the periosteum is and what it includes as to whether it is to be actively osteogenetic or not. If by chance the cleavage is deep as when the periosteum is removed with a sharp elevator and the bone scraped, the periosteum is very sure to be actively osteogenetic. On the other hand, if the periosteum is stripped off or removed with a blunt instrument, the cleavage is not likely to be deep enough to include the osteogenetic layer of cells on the periphery of the compact bone. In that instance the periosteum would constitute a connective tissue limiting membrane (Macewen) only and slight or no osteogenesis would occur.

12. It is believed that periosteum and marrow substance, on the bone-graft, serve an important rôle in aiding to establish an early and more abundant blood-supply from recipient bone to the transplant.

Other studies in bone growth, periosteal transplantation, etc., have been carried on, coincidently, as will appear.

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